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Dietary Tea Catechin Inclusion Changes Plasma Biochemical Parameters, Hormone Concentrations and Glutathione Redox Status in Goats

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ABSTRACT : The beneficial effects of tea catechins (TCs) are related not only to their antioxidant potential but also to the improvement of animal meat quality. In this study, we assessed the effects of dietary TC supplementation on plasma biochemical parameters, hormone responses, and glutathione redox status in goats. Forty Liuyang goats were randomly divided into four equal groups (10 animals/group) that were assigned to four experimental diets with TC supplementation at 4 levels (0, 2,000, 3,000 or 4,000 mg TC/kg DM feed). After a 60-day feeding trial, all goats were slaughtered and sampled. Dietary TC treatment had no significant effect on blood biochemical parameters, however, low-density lipoprotein cholesterol ($p<0.001$), triglyceride ($p<0.01$), plasma urea nitrogen ($p<0.01$), and glucose ($p<0.001$) decreased and total protein ($p<0.01$) and albumin ($p<0.05$) increased with the feeding time extension, and day 20 was the turning point for most of changes. Interactions were found in glutathione ($p<0.001$) and the ratio of reduced and oxidized glutathione ($p<0.05$) in whole blood between treatment and feeding time. Oxidized glutathione in blood was reduced ($p<0.05$) by 2,000 mg TC/kg feed supplementation, and a similar result was observed in *longissimus dorsi* muscle. Though plasma glutathione peroxidase ($p<0.01$) and glutathione reductase ($p<0.05$) activities were affected by treatment and feeding time interactions, and glutathione S-transferases activity increased with feeding day extension, no changed values appeared in *longissimus dorsi* muscle. In conclusion, dietary TC supplementation affected the concentrations of some blood metabolites and accelerated GSH depletion in the blood of goats. In terms of less high-density lipoprotein cholesterol, the highest insulin and IGF-I concentrations, the highest ratio of reduced and oxidized glutathione in plasma, the dosage of 2,000 mg TC/kg feed might be desirable for growing goats to prevent glutathione depletion and keep normal physiological metabolism. (**Key Words :** Plasma, Tea, Catechin, Biochemical and Hormone Parameters, Glutathione Redox, Goats)

INTRODUCTION

Oxidative stress, caused by excessive reactive oxygen species produced as the result of physiological aerobic metabolism of animals, can lead to the onset of various diseases, such as cancer, hypertension, diabetes, and atherosclerosis (Zelko et al., 2002; Rosenblat et al., 2008). The occurrence of all these diseases is related to blood metabolites, including total cholesterol (TCHO), triacylglycerols (TG) and glucose (GLU), and to the oxidation of TCHO in humans (Liu et al., 1992). In particular, low-density lipoprotein cholesterol (LDL-C) has been shown to be closely related to the pathogenesis of human atherosclerosis (Steinberg and Witztum, 2002).

Living cells of animals possess wide arrays of endogenous enzymatic and nonenzymatic antioxidant systems that function to prevent oxidative stress. Glutathione, a tripeptide found in mammalian cells, (GSH, γ-L-glutamyl-L-cysteinylglycine) is an important endogenous nonenzymatic antioxidant in the systems of cellular defense against oxidative stress (Cotgreave and Gerdes, 1998). Another potential role of high levels of GSH

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in muscle is to improve the nutritive value of meat and, therefore, to improve meat quality traits (Tang et al., 2003). GSH is beneficial because it helps maintain the redox potential in cells by scavenging reactive oxygen species (Cooper and Kristal, 1997). The reduced GSH reacts directly with reactive oxygen species in nonenzymatic reactions or acts as an electron donor (Winterbourn and Metodiewa, 1994). Its protective action is based on oxidation of the thiol group of its cysteine with the formation of oxidized glutathione (GSSG) (Tzeng et al., 1994). Recently, research has increasingly recognized that the amounts of GSH and GSSG and the ratio of GSH to GSSG are reliable indices of redox status and the extent of oxidative stress. Under normal physiological conditions, the GSH concentration is mainly controlled by glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glutathione S-transferases (GST) (Cnubben et al., 2001).

To maintain animal health and produce high-quality meat to meet consumer demand, researchers have recommended a dietary antioxidant supplementation strategy to alleviate oxidative stress and prevent GSH depletion in animals. Over the past several decades, animal nutrition researchers have focused on the functions of antioxidative vitamins, such as vitamins E and C (Malet, 1985; Minka et al., 2009), on animal health. However, high dietary concentrations of vitamins can be deleterious to animals because excessive vitamins will be catabolized or excreted (Aurousseau, 2002). Therefore, alternative strategy of natural plant-derived polyphenols supplementation in diet to modulate physiological metabolism of animals has been suggested (Anthony et al., 1998; Hsu et al., 2003).

Tea catechins (TCs), natural plant-extracted antioxidants, are of great interest for dietary supplementation in small ruminants (Sgorlon et al., 2005). Dietary TC supplementation can lower blood pressure (Henry, 1984) and can affect cellular oxidation (Ikeda et al., 1992) and cholesterol absorption (Riesmersma et al., 2001). Human studies have also reported that oral administration of green tea can decrease plasma TCHO, TG, and GLU concentrations (Sabu et al., 2002; Raederstoff et al., 2003). The underlying physiological mechanism of these effects was due mainly to the antioxidative properties of TC. The antioxidative and free-radical scavenging properties of TCs were controlled by their individual structures and by the numbers of active hydroxyl groups in the galloyl group (Hashim et al., 2005). Previously, the antioxidant functions of dietary TCs have been studied in humans and animals (He and Shahidi, 1997; Liu et al., 2000; Shirai and Suzuki, 2003; Mason et al., 2005; Tang et al., 2006; Periasamy et al., 2007). Generally, these studies have involved *in vivo* or *in vitro* trials. Furthermore, data from our laboratory have shown that dietary TC supplementation could decrease lipid oxidation and improve the meat quality of goats (Zhong et al., 2009). However, little attention has been paid to the effects of dietary TC supplementation on changes in blood metabolites in goats. This study was conducted to investigate the effects of different levels of dietary TC supplementation on blood biochemical parameters and hormone levels. The study also sought to clarify the effects of dietary TC supplementation on the endogenous GSH redox status in the blood and muscle of goats under normal feeding procedures.

MATERIALS AND METHODS

Preparation of tea catechins

The TCs (purity of 80.11%) were isolated from green tea leaves (*Camellia sinensis L.*) using high- pressure liquid chromatography (Model Waters 600, Waters Corp., Milford, USA) according to the procedure reported previously by Nonaka et al. (1983). The extracted TCs contained (+)-catechin and (-)-catechin (1.61%), (-)-epicatechin (5.77%), (-)-epigallocatechin (0.47%), (-)-epicatechin gallate (13.03%), (-)-gallocatechin gallate (1.56%), and (-)-epigallocatechin gallate (57.67%).

Experimental design, animal management and diets

The experiment was conducted according to the animal care and use guidelines of the Animal Care Committee, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China.

Forty Liuyang black male goats (a local breed) with average age of 8 months±10 days, an average initial body weight of 16.2±1.2 kg and the same genetic background were randomly divided into 4 equal groups (ten animals in each group) and were assigned to 4 experimental diets for a 60-day feeding trial. The control group (TC0) was fed a commercial basal diet without TC supplementation. The other three groups were fed the basal diet with dietary TC supplementation at levels of 2,000 (TC2000), 3,000 (TC3000) or 4,000 (TC4000) mg TC/kg feed (on a DM basis). The ingredients and chemical composition of the basal diet, formulated according to NRC (1981), are shown in Table 1. The animals were reared at the Animal Study Centre of Institute of Subtropical Agriculture. All goats were castrated surgically before the trial and then allowed to recover for two weeks. During the trial, each goat was fed twice daily (08:00 and 20:00 h) with 578 g feed per day (on DM basis) to meet nutrient requirements. Orts were recorded daily. Each goat was assigned to an individual finishing barn with an average temperature of 24±1°C and free access to fresh water. After a 60-day feeding period, all goats were humanely slaughtered, and samples were collected.

Table 1. Ingredients and chemical composition of the basal experimental diet

Ingredient	% DM
Maize stover	45
Ground corn	25
Soybean meal	15
Wheat bran	11.2
Urea	0.2
Calcium carbonate	0.9
Calcium bicarbonate	0.6
Sodium chloride	0.6
Mineral and vitamin salts [1]	1.5
Chemical composition	
DM (%)	89.8
OM (% DM)	92.7
ME (Mcal/kg DM) [2]	2.75
CP (% DM)	12.3
NDF (% DM)	36.7
ADF (% DM)	24.2
Ca (% DM)	0.92
Total P (% DM)	0.74

[1] Contained per kg: 227 g $MgSO_4 \cdot H_2O$, 12.5 g $FeSO_4 \cdot 7H_2O$, 2.8 g $CuSO_4 \cdot 5H_2O$, 12.2 g $MnSO_4 \cdot H_2O$, 13.4 g $ZnSO_4 \cdot H_2O$, 20 mg Na_2SeO_3, 50 mg KI, 35 mg $CoCl_2 \cdot 6H_2O$, 90,000 IU vitamin A, 17,000 IU vitamin D, and 17,500 IU vitamin E.

[2] Metabolic energy was calculated according to NRC (1981).

Blood and muscle sampling procedures

At days 0, 20, 40 and 60 of the trial period, 5 ml blood was taken from the jugular vein of each goat and placed in aseptic vacutainer tubes. GSH and GSSG levels were analyzed immediately. Two additional 20 ml blood samples were taken from the jugular vein of each goat at 06:00 and 10:00 h and were placed in aseptic vacutainer tubes containing Li-heparin (Becton Dickinon, Vacutainer Systems, Rutherford, NJ). Blood samples were then centrifuged using a low-temperature centrifuge (Himac CR22G2, Hitachi Koki Co., Ltd) at 3,000 rpm for 15 min at 4°C to harvest plasma. Plasma samples were stored at -20°C until further analysis of biochemical parameters, hormone levels and GSH metabolic enzyme activity.

At the end of the 60-day feeding trial, all goats were individually weighed and humanely slaughtered using commercial procedures. The slaughtered animals were hung to remove the skin, head (at the occipitoatlantal joint), forefeet (at the carpal-metacarpal joint), hind feet (at the tarsal-metatarsal joint), gastrointestinal tract and visceral organs. The carcass was then chilled for 12 h at 4°C in cold storage. The left side of the carcass was subsequently used for muscle sampling. Approximately 50 g of *longissimus dorsi* (LD) muscle was removed from the left side of the carcass within 24 h postmortem, vacuum-packaged and stored at -20°C until further analysis of GSH metabolic enzyme activity and GSH and GSSG levels.

Analytical procedure

The plasma was used to determine total protein (TP), albumin (ALB), globulin (GLB), GLU, plasma urea nitrogen (PUN), TG, TCHO, LDL-C and high-density lipoprotein cholesterol (HDL-C) levels using an automatic biochemistry analyzer (SYNCHRON CX5 PRO, Beckman Coulter, USA). The growth hormone (GH), insulinoid growth factor I (IGF-I), insulinoid growth factor II (IGF-II), aldosterone (ALD), insulin and cortisol concentrations in plasma were analyzed using commercial kits (Jiancheng Biology Co., Nanjing, China) and a radiometer (HH6003A, Hehai Advance Technology Co., LTD, Beijing, China).

The GSH concentrations in blood and muscle samples were determined by the pre-column-phthalaldehyde (OPA) derivative method using HPLC as described by Cereser et al. (2001). The concentrations of GSH and GSSG were expressed as mg/g protein, and the total protein of LD was determined by the method of Lowry et al. (1951). The concentrations of GSH and GSSG were expressed as mg/L.

The activities of GSH-Px, GR and GST in plasma and LD were measured using previously reported methods (Glatzle et al., 1974; Habig et al., 1974; Chang et al., 1997) using commercially available kits (Jiancheng Biology Co., Nanjing, China). The GSH-Px and GST activities of plasma and muscle homogenate were measured spectrophotometrically and were expressed as U/ml. The GR activity was determined by monitoring the oxidation of NADPH spectrophotometrically at 340 nm and was expressed as U/L.

Statistical analysis

Blood parameters data were analyzed by SAS (2000) using the MIXED model procedure (Littell et al., 1996) with a model consisting of diet treatment, sampling time, and treatment×sampling time interaction. Means were separated using least squares mean and presented with the standard error of the mean (SEM) in tabular form. When the interaction was not significant but the main effects of treatment and/or sampling time were significant, difference among all means was tested separately with Duncan's multiple-range tests. Statistical significance was declared at $p \leq 0.05$.

Data on muscle GSH, GSH-Px, GR, and GST activities were analyzed using the GLM procedures of SAS (2002). Differences among all observed means were tested with Duncan's multiple-range tests. Statistical significance was set at $p \leq 0.05$. Orthogonal polynomial contrasts were used to examine the responses (linear, quadratic and cubic) to increased supplement levels of TC in the diets. In the orthogonal polynomial analysis, coefficients were corrected because of unequal spacing of treatments.

Table 2. Effect of dietary tea catechins supplementation on plasma biochemical parameters

Variable [1]	Treatment [2]				SEM	Sampling time				SEM	Significance [3]		
	TC0	TC2000	TC3000	TC4000		Day0	Day20	Day40	Day60		Treat	Day	Treat×Day
HDL-C (mmol/L)	1.92	1.69	1.93	1.88	0.146	1.84	1.91	1.76	1.91	0.090	NS	NS	NS
LDL-C (mmol/L)	0.40	0.42	0.36	0.36	0.020	0.47^{a}	0.37^{b}	0.37^{b}	0.35^{b}	0.021	NS	***	NS
TCHO (mmol/L)	1.70	1.58	1.69	1.68	0.148	1.73	1.62	1.56	1.74	0.089	NS	NS	NS
TG (mmol/L)	0.20	0.21	0.16	0.18	0.020	0.24^{a}	0.20^{ab}	0.16^{b}	0.16^{b}	0.018	NS	***	NS
TP (g/L)	70.2	72.2	66.8	67.5	2.01	67.0^{b}	72.6^{a}	68.5^{b}	68.6^{b}	1.29	NS	**	NS
ALB (g/L)	22.6	23.4	23.9	25.0	1.34	23.1	23.3	23.4	25.0	0.76	NS	*	NS
GLB (g/L)	48.0	49.3	42.9	45.7	0.91	47.3	46.6	44.1	41.5	0.73	NS	NS	NS
PUN (mmol/L)	8.29	8.01	8.32	8.24	0.255	9.21^{a}	8.47^{ab}	7.91^{bc}	7.26^{c}	0.263	NS	**	NS
GLU (mmol/L)	2.83	3.02	3.20	3.38	0.162	3.20^{a}	3.24^{a}	2.77^{b}	3.21^{a}	0.109	NS	***	NS

^{a,b,c} Mean values followed by different superscripts in the same row of sampling time effect differ significantly ($p<0.05$).
[1] HDL-C = High-density lipoprotein cholesterol; LDL-C = Low-density lipoprotein cholesterol; TCHO = Total cholesterol; TG = Triglyceride; TP = Total plasma protein; ALB = Albumin; GLB = Globulin; PUN = Plasma urea nitrogen; GLU = Glucose.
[2] TC0 = No TC; TC2000 = 2,000 mg TC/kg feed; TC3000 = 3,000 mg TC/kg feed; TC4000 = 4,000 mg TC/kg feed.
[3] NS = Not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

RESULTS

Plasma biochemical parameters and hormones

Plasma biochemical parameters are presented in Table 2. Dietary TC treatment had no significant effect on all parameters ($p>0.05$). However, LDL-C ($p<0.001$), TG ($p<0.001$), PUN ($p<0.01$), and GLU ($p<0.001$) were reduced and TP ($p<0.01$) increased with the feeding time extension.

The measured plasma hormone concentrations are listed in Table 3. Tea catechins treatment had no significant effect on insulin concentration, and the value in TC3000 treatment was lowest among treatments, however, the concentration in day 20 was increased ($p<0.001$). There was an interaction in IGF-II ($p<0.001$) between treatment and feeding time, which appeared a threshold in TC2000 and day 20. There was an interaction in cortisol level ($p<0.001$) between treatment and feeding time, which appeared largely because of increased dosage and extended feeding time. The plasma GH, IGF-I, and ALD concentrations did not differ among four treatments.

GSH status in whole blood and muscle homogenate

The GSH statuses in whole blood and muscle homogenate are given in Table 4 and 5, respectively. Though GSH concentration in muscle homogenate did not change, value in whole blood was affected by interaction between treatment and feeding time ($p<0.001$), and it decreased with dietary TC level increasing but increased with feeding time extension. The GSSG concentration in whole blood was affected by treatment ($p<0.05$) and feeding time ($p<0.001$), and the value in muscle homogenate showed similar changed tendency ($p<0.05$) with whole blood. TC2000 had lowest GSSG concentration and highest GSH:GSSG in whole blood and muscle homogenate when compared with other treatments.

GSH metabolic enzymes in plasma and muscle homogenate

The GSH metabolic enzyme activities in plasma and muscle homogenate are presented in Table 4 and 5, respectively. There were interactions in GSH-Px ($p<0.01$)

Table 3. Effect of dietary tea catechins supplementation on plasma hormone concentrations in goats

Variable[1]	Treatment [2]				SEM	Sampling Time				SEM	Significance [3]		
	TC0	TC2000	TC3000	TC4000		Day0	Day20	Day40	Day60		Treat	Day	Treat×Day
GH (ng/ml)	0.64	0.70	0.64	0.68	0.023	0.68	0.66	0.68	0.65	0.017	NS	NS	NS
Insulin (IU/ml)	21.2	24.6	20.2	22.7	1.33	17.4^{b}	34.6^{a}	18.2^{b}	18.4^{b}	1.01	NS	***	NS
IGF- I (ng/ml)	313	355	306	240	125.1	375	262	302	373	83.7	NS	NS	NS
IGF- II (pg/ml)	55.4	59.0	41.9	43.3	4.16	48.6	65.5	43.3	47.5	4.70	*	**	***
Cortisol (ng/ml)	334	210	257	235	10.7	320	351	168	197	17.6	***	***	***
ALD (ng/ml)	0.03	0.02	0.03	0.03	0.007	0.03^{ab}	0.02^{b}	0.03^{b}	0.05^{a}	0.006	NS	NS	NS

^{a,b} Mean values followed by different superscripts in the same row of treatment or sampling time effect differ significantly ($p<0.05$).
[1] GH = Growth hormone; IGF-I = Insulinoid growth factor I; IGF-II = Insulinoid growth factor II; ALD = Aldosterone.
[2] TC0 = No TC; TC2000 = 2,000 mg TC/kg feed; TC3000 = 3,000 mg TC/kg feed; TC4000 = 4,000 mg TC/kg feed.
[3] L = Linear; Q = Quadratic; C = Cubic; NS = Not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 4. Effect of dietary tea catechins supplementation on glutathione redox status and glutathione metabolic enzyme activity in the plasma of goats

Variable [1]	Treatment [2]				SEM	Sampling time				SEM	Significance [3]		
	TC0	TC2000	TC3000	TC4000		Day0	Day20	Day40	Day60		Treat	Day	Treat×Day
GSH redox status													
GSH (mg/L)	2.37	2.06	2.00	1.78	0.113	1.09	1.36	1.76	4.00	0.130	*	***	***
GSSG (mg/L)	0.20^{a}	0.18^{b}	0.19^{a}	0.20^{a}	0.007	0.10^{d}	0.13^{c}	0.16^{b}	0.39^{a}	0.007	*	***	NS
GSH:GSSG	10.7	11.5	10.3	7.8	0.50	11.5	11.1	10.9	10.2	0.57	*	NS	*
Enzyme activity													
GSH-Px (U/ml)	305	305	236	266	13.9	257	296	302	256	10.5	**	***	**
GR (U/L)	12.7	12.8	12.7	11.0	0.56	11.3	12.8	13.4	11.7	0.62	*	*	*
GST (U/ml)	9.10	8.47	9.38	9.19	0.415	4.36^{c}	7.80^{b}	11.74^{a}	12.23^{a}	0.307	NS	***	NS

^{a,b,c,d} Mean values followed by different superscripts in the same row of treatment or sampling time effect differ significantly ($p<0.05$).
[1] GSH = Reduced glutathione; GSSG = Oxidized glutathione; GSH-Px = Glutathione peroxidase; GR = Glutathione reductase; GST = Glutathione S-transferases.
[2] TC0 = No TC; TC2000 = 2,000 mg TC/kg feed; TC3000 = 3,000 mg TC/kg feed; TC4000 = 4,000 mg TC/kg feed.
[3] NS = Not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

and GR ($p<0.05$) activities between treatment and feeding time. The GST activity in plasma was increased by feeding time extending ($p<0.001$). Nonetheless, all measured enzyme activities did not change in muscle homogenate at end of the experiment.

DISCUSSION

Catechins are reported to inhibit the absorption of TCHO and to promote TCHO excretion (Ikeda et al., 1992). Epidemiological studies in humans have demonstrated that a higher content of plant polyphenols in the diet decreases the occurrence of coronary heart disease by decreasing blood lipid content, especially TG and TCHO (Vinson et al., 1995; Hara, 2001). However, our data showed that plasma TG and TCHO levels were not affected by TC supplementation in the diets of goats. The reason for this result might be that although plasma LDL-C decreased with feeding time extension and dosage of TC, the fluctuated change of HDL-C might cancel out their TCHO transport function. Another explanation might be that metabolic patterns differ between humans and ruminants. Generally speaking, higher HDL-C and lower LDL-C concentrations in plasma are beneficial in that they efficiently decrease the TCHO level in blood (Fielding and Fielding, 1995). The results of studies agree with the previous results reported by Shirai and Suzuki (2003) showing that the plasma TG level in the higher TC dose group was lower than that of the control group, although there was no significant difference between them. Accordingly, we inferred that dietary TC supplementation had no effect on the plasma TCHO and TG metabolism of goats.

In this study, we observed that the TC2000 group exhibited higher plasma insulin and IGF-I concentrations when compared with other groups. However, the plasma GLU levels in all TC treatment groups were higher than that

Table 5. Effect of dietary tea catechins supplementation on glutathione redox status and glutathione metabolic enzyme activity in the muscle homogenate of goats

Variable [1]	Treatment [2]				SEM	TC effect (p-value)[3]		
	TC0	TC2000	TC3000	TC4000		L	Q	C
GSH redox status								
GSH (mg/g protein)	3.10	4.31	3.61	4.09	0.419	NS	NS	NS
GSSG (mg/g protein)	0.35^{ab}	0.33^{b}	0.39^{ab}	0.44^{a}	0.028	*	NS	NS
GSH:GSSG	8.89^{b}	13.01^{a}	9.32^{b}	9.36^{b}	0.764	NS	**	**
Enzyme activity								
GSH-Px (U/ml)	12.0	12.8	8.6	9.1	1.39	NS	NS	NS
GR (U/L)	5.84	7.05	6.18	8.96	1.026	NS	NS	NS
GST (U/ml)	15.8	15.6	11.2	11.8	1.71	NS	NS	NS

^{a,b} Mean values followed by different superscripts in the same row differ significantly ($p<0.05$).
[1] GSH = Reduced glutathione; GSSG = Oxidized glutathione; GSH-Px = Glutathione peroxidase; GR = Glutathione reductase; GST = Glutathione S-transferases.
[2] TC0 = No TC; TC2000 = 2,000 mg TC/kg feed; TC3000 = 3,000 mg TC/kg feed; TC4000 = 4,000 mg TC/kg feed.
[3] L = Linear; Q = Quadratic; C = Cubic; NS = Not significant; * $p<0.05$; ** $p<0.01$.

of the control group. This result suggests that dietary TC supplementation might improve the catabolism of carbohydrates under normal physiological conditions in goats. In contrast to our findings, Khan et al. (2007) reported that dietary green tea supplementation decreased the serum GLU concentration of rats but that it enhanced the activities of carbohydrate metabolism enzymes in rats. The reason for this difference might be that different animal species show different responses to dietary TC consumption. Our results show that the plasma TP and ALB level increased with feeding time extension but that the plasma GLB concentration was not affected by TC supplementation. Although our previous work reported that average daily gain of goats in TC3000 and TC4000 increased (Tan et al., 2010), plasma GLB in the these two groups was lower than in the control and TC2000 groups and a long time of feeding also resulted in lower GLB value than control. This decrease did not facilitate higher immunity and was therefore not advantageous for animals. The reason for these changes might be the pro-oxidant action and the direct interaction of excessive catechins with target proteins (Galati and O'Brien, 2004; Tachibana et al, 2004; Yang et al., 2009). The current findings therefore imply that the TC supplementation dose under 2,000 mg/kg DM in the diets of goats is benefit for overall normal blood constituent metabolism.

To evaluate the effect of TC supplementation on the stress extent of goats, we investigated the plasma cortisol response. We observed that dietary TC supplementation decreased the plasma cortisol level. This finding suggests that the addition of dietary TC has potential to moderate oxidative stress because elevated plasma cortisol concentrations indicate higher stress levels in goats and have adverse effects on physiological functions (Kannan et al., 2007). Very little information about the plasma cortisol response to antioxidant consumption is found in the literature. However, some previous studies have demonstrated that dietary antioxidant supplementation does not influence the plasma cortisol concentration in goats under preslaughter stress (Galipalli et al., 2004; Kannan et al., 2007). Additional studies are needed to explore the relationship between antioxidant consumption and plasma cortisol.

Glutathione is the most abundant intracellular thiol-based antioxidant present in millimolar concentrations. It plays an important role in maintaining the primary defense capacity of cells (Nordberg and Arner, 2001; Das and Vasudevan, 2005). Levels of reduced GSH are within the range of 1 to 10 mM, and GSH:GSSG exceeds 100 in mammalian cells under normal physiological conditions, whereas the ratio of GSH to GSSG could be less than 100 under conditions of oxidative stress (Chai et al., 1994). Glutathione depletion and a decrease in the GSH:GSSG are linked to the pathogenesis of many diseases, such as lung cancer (Navarro et al., 1997), diabetes (Samiec et al., 1998) and conditions leading to coronary heart surgeries (Pantke et al., 1999). Hultberg and Hultberg (2006) reported that vitamin C increased the total GSH amount but that only lower doses of plant-extracted flavonoids increased total GSH amounts in human hepatoma cells. However, the present results demonstrated that, although the plasma GSH-Px level decreased, the level of reduced GSH still decreased with the increase of TC supplementation. Furthermore, the GSSG concentration and GSH:GSSG decreased. The results of the study suggest that TC supplementation accelerated endogenous GSH depletion. These results further indicate that reduced GSH was not oxidized into GSSG. Rather, it was probably combined with other compounds. Support for this hypothesis may be found in work by Na and Surh (2008). These authors suggested that oxidized or other reactive forms of catechins can conjugate with GSH and thereby decrease the cellular GSH level.

The change in the GSH redox status in LD was different from that of plasma. The results of the current study showed that lower doses of TC supplementation could increase the reduced GSH level and significantly increase GSH:GSSG. This beneficial effect could serve to improve meat quality. The higher amounts of reduced GSH could act as an antioxidant and could be available to participate in redox reactions. *In vitro* experiments have shown that GSH can slow the oxidation of bovine myoglobin (Tang et al., 2003). Additionally, the high reducing potential of GSH facilitates the regeneration of other antioxidants (Jones, 2006), e.g., vitamins E and C (Meister and Anderson, 1983; Li et al., 2001). This effect further enhances the antioxidant defense capacity of meat. The improvement of meat quality has also been identified by our previous research (Zhong et al., 2009). The increased GSH:GSSG in LD associated with lower doses of TC supplementation implies that the effects of dietary TC supplementation are dose-dependent for scavenging ROS (Granado-Serrano et al., 2009).

The GSH content is mainly controlled by GSH-Px, GR and GST (Cnubben et al., 2001). Tea polyphenols are known to induce phase 2 antioxidant enzymes and therefore to increase the biosynthesis of antioxidant and detoxification enzymes and major cellular antioxidants, especially GSH (Chou et al., 2000). However, our data indicate that GSH was regulated not only by enzyme-controlled pathways when the diet was supplemented with TC in goats. The basis for this argument is that TC supplementation had no significant effect on GR and GST activity in plasma and muscle (other than decreased GSH-Px activity in plasma). On the contrary, Zhang et al. (1997) reported that plant polyphenols exhibited varying degrees of concentration-dependent inhibition of GR activity. This

difference might result from the different supplementation doses and experimental animal models. Several studies have also reported that plant polyphenols inhibit GST activity (Zhang and Das, 1994; Çoruh et al., 2007). However, Na and Surh (2008) also reported that reactive gallocatechin gallate derivatives reduced the cellular GSH level but increased the expression of detoxifying enzymes. This finding is supported by our current results. We suggest that inhibition of GST activity under *in vivo* conditions is concentration dependent. A lower dose of dietary TC supplementation in goats would therefore exert its antioxidant capacity by inhibiting GST activity and slowing GSH depletion.

CONCLUSIONS

This study reports three important findings. i) Dietary tea catechins supplementation did not affect the total cholestrol and triacylglycerols concentrations in the plasma of goats. ii) Dietary tea catechins reduced plasma cortisol levels and thereby protected goats against stress, but higher doses of dietary tea catechins (over 3,000 mg/kg) were likely to decrease the immunity of goats by decreasing plasma total protein and globulin levels. iii) Dietary TC supplementation accelerated GSH depletion in the plasma of goats.

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